



# Analysis of Anticoagulant Drugs by HPLC

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## Abstract

Warfarin is used in human medicine as anticoagulant to decrease the rate of blood clotting. This helps to prevent thrombosis after operations. In contrast, warfarin is also used as a rodenticide against rats, making use of the same effect – here the animals die because blood clotting is prevented.

Figure 1 shows the chromatogram of warfarin using gradient analysis on a reversed phase column and UV detection. To avoid decomposition of samples the autosampler temperature was set to 4 °C.

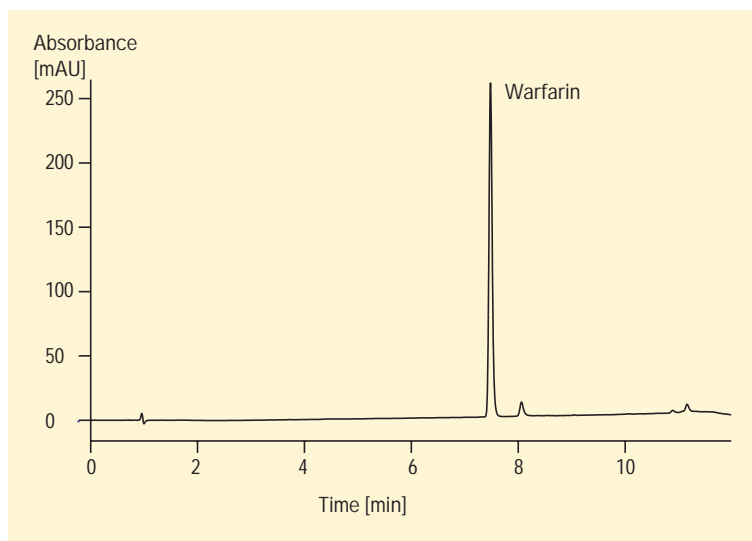


Figure 1  
Analysis of wafarin using a variable wavelength detector

## Conditions

### Column

4.6 x 75 mm Zorbax SB-C18, 3.5 µm

### Mobile phase

A = 0.025 M  $\text{KH}_2\text{PO}_4$  in water  
(pH = 3 with  $\text{H}_2\text{SO}_4$ )

B = acetonitrile

**Flow rate** 1.0 ml/min

**Gradient** 20 % B to 80 % B in 10 min

### Column wash

80 % B to 20 % B in 2 min

### UV detector

variable wavelength detector

204 nm, standard cell

fluorescence detector

272/355 nm, standard cell

**Column compartment temperature**  
25 °C

**Stop time** 12 min

**Post time** 5 min

**Injection volume** 5 µl



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The performance of the HPLC method is shown in the table below.

Compound	LOD for S/N=2 (mg/l)*	Precision of RT (RSD of 10 runs) (100 mg/l)*	Precision of Area (RSD of 10 runs) (100 mg/l)*
Warfarin	0.1	0.02	0.46
* Injection volume: 5 µl			

The analysis can also be done using the Agilent 1100 Series fluorescence detector (figure 2).

The method presented here shows an easy but reliable and precise analysis of the anticoagulant drug warfarin. The values for LOD, precision of RT and precision of area show the good performance of the analysis.

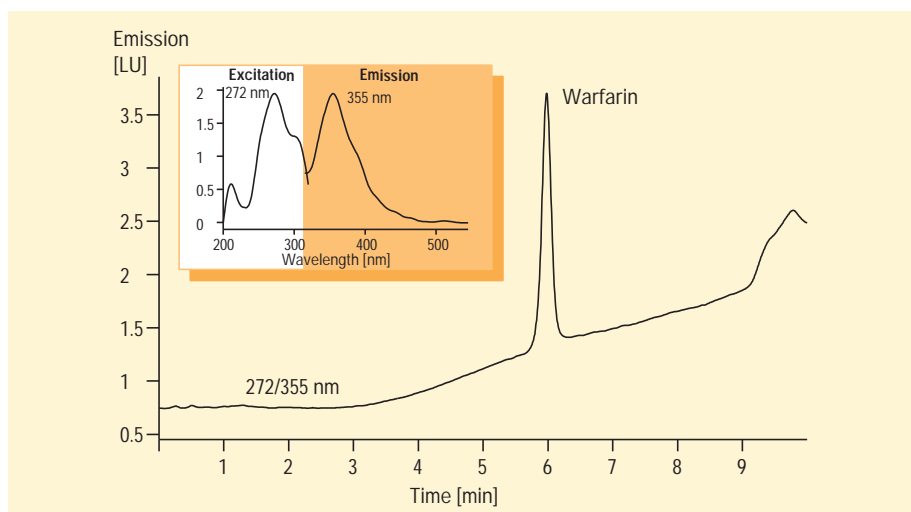


Figure 2  
Analysis of warfarin using a fluorescence detector  
(column: Zorbax SB-C18, 2.1 x 50 mm, 5 µm)

**Note:**

Since the method was specifically developed on the Agilent 1100 Series system you might not be able to reproduce this analysis on an older system or even on a new system with lower performance. To avoid sample decomposition it is necessary to use a cooled autosampler, for example, the Agilent 1100 Series thermostatted autosampler. The analysis can be performed with either an Agilent 1100 Series variable wavelength detector or an Agilent 1100 Series fluorescence detector.

## Equipment

### Agilent 1100 Series

- Quaternary pump (includes vacuum degasser)
- Thermostatted autosampler
- Thermostatted column compartment
- Variable wavelength detector, standard flow cell, 10-mm path length, 13-µl cell volume

### Alternative:

- Vacuum degasser
- Binary pump
- Diode-array detector, standard flow cell 10-mm path length, 13-µl cell volume
- Fluorescence detector, standard flow cell, 8-µl cell volume
- Agilent ChemStation + 3D software

### Columns

- Zorbax SB-C18, 3.5 µm, 4.6 x 75 mm (Agilent part number 866953-902)
- *Recommended:* Guard cartridges Zorbax SB-C18, 5 µm, 4.6 x 12.5 mm (Agilent part number 820950-920, 4/pk)

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